INFECTION AND IMMUNITY, Jan. 1983, p. 1–6
0019-9567/83/010001-06$02.00/0

Vol. 39, No. 1

Evidence for Reactive Oxygen Intermediates Causing Hemolysis and Parasite Death in Malaria

I. A. CLARK* AND N. H. HUNT

John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory 2601, Australia

Received 6 July 1982/Accepted 5 October 1982

A rapid reduction in parasitemia associated with damage to intraerythrocytic parasites was observed in *Plasmodium vinckei*-infected mice after they had received a single intravenous injection of alloxan. This was not prevented by prior injection of glucose, but was prevented by desferrioxamine or diethyldithiocarbamate. Prior injection of propanol partially blocked the phenomenon. A transient hemolysis was observed in malaria-infected mice, but not in controls, after injection of alloxan. This was also blocked by desferrioxamine, but not by glucose. Both the fall in parasitemia and hemolysis occurred, but less dramatically, when phenylhydrazine or hydrogen peroxide was injected into parasitized mice. Again, the hemolysis was blocked by desferroxamine. These observations are consistent with the parasite death and hemolysis being mediated by reactive oxygen species, possibly hydroxyl radicals, and have implications for our understanding of hemolysis, endothelial damage, and parasite suppression in acute malaria. Our evidence that malaria parasites are susceptible to free oxygen radicals supports the view that high intraerythrocytic oxidative stress may contribute to the high frequencies in malarial areas of genes for certain erythrocyte-related traits and suggests that some antimalarial drugs may suppress parasites partly through oxidative damage.

Acute malaria is a complex disease, with much pathology not easily attributable to the presence of protozoa inside circulating erythrocytes. We have proposed that release of soluble mediators from mononuclear phagocytes may explain many of these changes and also the concurrent suppression of the parasites (2). This model includes release of arachidonate metabolites (3, 6; manuscript in preparation), as do current explanations for damage to vascular endothelial cells in sepsis (44), hypertension (26), and trauma (49). Because these authors argue that oxygen-derived free radicals released during the formation of arachidonate metabolites might mediate this endothelial cell damage, we investigated whether this family of reactive oxygen species could also cause pathology and parasite inhibition associated with acute malaria. We were encouraged by evidence that erythrocyte membranes (28) and endothelial cells (25), both of which are damaged in malaria, are very susceptible to damage by free radicals, and by reports that other protozoan parasites are susceptible to toxic oxygen species (33, 36). We have found that when alloxan is injected into *Plasmodium vinckei*-infected mice there is a transient hemolysis, and the parasites are rapidly damaged. In keeping with arguments on the nature of alloxan-induced hemolysis of tocopherol-deficient erythrocytes and damage to islet β-cells, it seems likely that reactive oxygen species mediate these changes.

MATERIALS AND METHODS

Mice and parasites. CBA/CaH mice of either sex and between 6 and 10 weeks old were used in these experiments. Neither sex nor age had an apparent effect on the outcome. *P. vinckei* subsp. *vinckei* (strain V52, from F. E. G. Cox) was stored at −96°C and before use had been passaged several times in CBA/CaH mice. All infections were initiated by the intraperitoneal injection of 10^6 parasitized erythrocytes and monitored by examining Giemsa-stained thin smears.

Reagents. Alloxan monohydrate (Sigma Chemical Co.) was dissolved in normal saline at 12.5 mg/ml, stored in the dark at 4°C, and injected without further dilution. This remained stable for at least 6 weeks. Freeze-dried desferrioxamine (Desferal, Ciba Pharmaceuticals) was used within 1 week of reconstitution with distilled water to 100 mg/ml, having been stored at 4°C. Diethyldithiocarbamate (DDC) (Sigma Chemical Co.), *n*-propanol, hydrogen peroxide, glucose (BDH Chemicals Ltd.), and phenylhydrazine hydrochloride (Fluka AG) were freshly dissolved in saline before each experiment. Desferrioxamine was injected at stock concentration, and other reagents were used as follows: DDC as 200 μl of a 75-mg/ml solution, *n*-propanol as 500 μl of a 10% (vol/vol) solution, hydro-

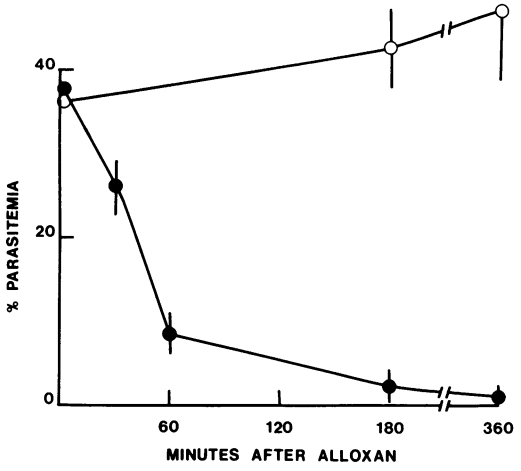

FIG. 1. Effect of intravenous injection of alloxan (50 mg/kg) on *P. vinckei* infections. Symbols: (●) alloxan; (○) saline. Standard deviations are indicated by vertical bars; $n = 35$ mice at each point.

gen peroxide as 200 μl of a 1% (vol/vol) solution, glucose as 200 μl of a 250-mg/ml solution, and phenylhydrazine as 100 or 200 μl of a 75-mg/ml solution. The stock hydrogen peroxide solution was 30% (wt/vol).

Hematocrits were measured after a 5-min spin in a Clay Adams Readacrit centrifuge.

RESULTS

Injection of alloxan into malaria-infected mice. On day 6 after infection with *P. vinckei*, when parasitemias were between 30 and 60%, mice received an intravenous injection of alloxan. Figure 1 shows the outcome of seven experiments with an alloxan dose of 50 mg/ml. Doses ranging from 25 to 100 mg/kg gave very similar results, and two or three doses of 50 mg/kg each several days apart, when monitored for a further month, seemed to have cured the infections completely (data not shown). The effects were clearly evident within 30 min of injection, when blood smears contained many degenerating intraerythrocytic parasites.

These effects were independent of the diabetogenic action of alloxan since injection of glucose immediately beforehand, which prevents alloxan from causing diabetes (30), did not inhibit its action against parasites (Fig. 2). Even without glucose injections, the fluctuations in blood glucose levels were minor when parasite damage occurred. In contrast, pretreatment with desferroxamine, a specific iron chelator which inhibits formation of hydroxyl radicals from superoxide and hydrogen peroxide by the iron-catalyzed Haber-Weiss reaction (21), completely prevented the antimalarial activity of alloxan. When 200 mg of desferroxamine per kg was injected intraperitoneally 30 min before alloxan, the parasites (Fig. 2) remained intact and apparently healthy. This effect was total, without seeming to harm the mice, with doses between 40 and 800 mg/kg (data not shown).

Alloxan-induced chemiluminescence in a cell-free system appears to be a hydroxyl radical-mediated phenomenon, and the avid metal chelator DDC is as inhibitory as desferrioxamine in this system (20). At all doses we tested (0.3 to 1.2 g/kg), pretreatment with DDC totally inhibited the action of alloxan against malaria parasites (Fig. 2, 0.6 g/kg shown). Figure 2 also shows that pretreatment with propanol (1.6 g/kg) partially inhibited the killing of malarial parasites by alloxan. Propanol prevents alloxan diabetes, probably by scavenging hydroxyl radicals (23). Neither desferroxamine, DDC, nor propanol alone had any apparent effect on parasites over a 3-h period.

A transient hemolysis, evidenced by hemoglobinuria and lowered hematocrits, accompanied the damage to parasites. Hemolysis was not seen in normal mice given the same dose of alloxan. However, parasite damage was not dependent on hemolysis since many degenerating intraerythrocytic parasites were present (30 to 40 per field). A comparison of parasitemia and degree of hemolysis, together with the presence of degenerating parasites within intact erythrocytes, indicated that hemolysis was not confined to parasitized erythrocytes. Marked hemoglobinuria 30 min after alloxan injection indicated

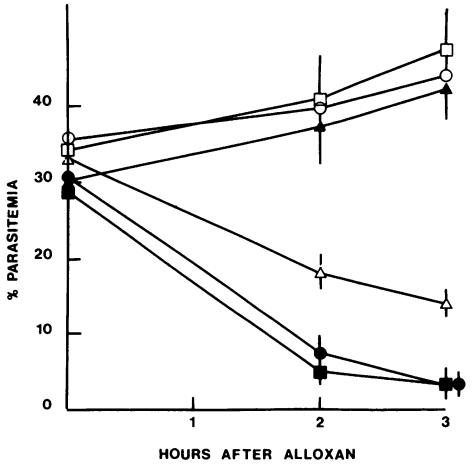

FIG. 2. Effect of pretreatment with glucose, propanol, desferrioxamine, or DDC on antimalarial action of alloxan (50 mg/kg, intravenous). Symbols: (■) D-glucose (2 g/kg) given intravenously immediately before alloxan; (△) *n*-propanol (1.6 g/kg), (▲) desferrioxamine (200 mg/kg), or (□) DDC (0.6 g/kg) given intraperitoneally 30 min before alloxan; (●) alloxan alone; (○) saline. Standard deviations are indicated by vertical bars; $n = 5$ at each point. Representative outcome of two to four experiments is shown.

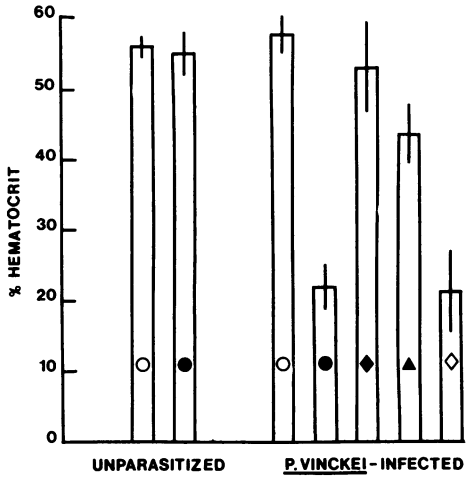

FIG. 3. Hematocrit (2 h after alloxan injection) of unparasitized mice and others with 41 ± 7% parasitemia of *P. vinckei*. Symbols: (○) controls; (●) intravenous alloxan (50 mg/kg) alone; (◆) intraperitoneal desferrioxamine (200 mg/kg) alone; (▲) desferrioxamine given 30 min before alloxan; (◇) intravenous glucose (2 g/kg) given immediately before alloxan. Standard deviations are indicated by vertical bars; n = 5 for each group. Representative outcome of three experiments is shown.

that by then most of the hemolysis reflected in the 2-h hematocrit values (Fig. 3) had already occurred. Figure 3 shows that, as noted for the antimalarial action of alloxan, its hemolytic activity was blocked by desferrioxamine, but not by glucose. It was clear from the urine color that DDC, like desferrioxamine, prevented alloxan-induced hemolysis.

Injection of phenylhydrazine or hydrogen peroxide into malaria-infected mice. Mice with 15 to 40% *P. vinckei* parasitemia received subcutaneous injections of phenylhydrazine. Four injections of 30 to 60 mg/kg each were given 12 h apart. Other groups received four intravenous injections of 200 μl 1% hydrogen peroxide every 12 h. A transient hemoglobinuria, which, like that of alloxan, could be prevented by injecting desferrioxamine (200 mg/kg) 30 min beforehand, was observed after the first injection of phenylhydrazine or hydrogen peroxide. These dose rates produced no hemoglobinuria in uninfected mice. Figure 4 shows the change in parasitemia over the 4 days after the start of these injections. Parasites cleared entirely from the circulation of mice given the lower dose of phenylhydrazine, and mice receiving 60 mg/kg, after a more dramatic fall in parasitemia, died on day 2. Degenerated intraerythrocytic parasites were evident in erythrocytes on smears from both phenylhydrazine groups and those given hydrogen peroxide. Uninjected parasitized mice died, with high parasite densities, on day 3 after injections began. When we attempted to see whether desferrioxamine would inhibit phenylhydrazine or hydrogen peroxide-induced parasite suppression, we noted that during the time required to monitor these slower-acting agents, desferrioxamine alone inhibited the parasites, perhaps by limiting their nutritional iron. This is consistent with a recent report about desferrioxamine added to cultured *Plasmodium falciparum* (C. Raventos, S. Pollack, and R. L. Nagel, Clin. Res. **30**:377A, 1982).

DISCUSSION

Here we present evidence that alloxan will kill *P. vinckei* in vivo (Fig. 1) and, in the doses used, causes hemolysis in malaria-infected mice but not in controls (Fig. 3). Although most of the studies of alloxan concern its capacity to induce diabetes, alloxan also causes hemolysis in tocopherol-deficient rats (42) and produces an experimental pulmonary edema characterized by damage to vascular endothelial cells (8). Subsequent studies of this hemolysis incriminate toxic oxygen species (12, 43). The hemolysis we observed in malarial mice given alloxan (Fig. 3) implies that their erythrocytes, as in tocopherol deficiency (42), were already under oxidative stress. Recent evidence that the damage alloxan causes to islet β-cells is mediated by reactive oxygen species, probably hydroxyl radicals (14, 20, 22), is in agreement with its harmful effects

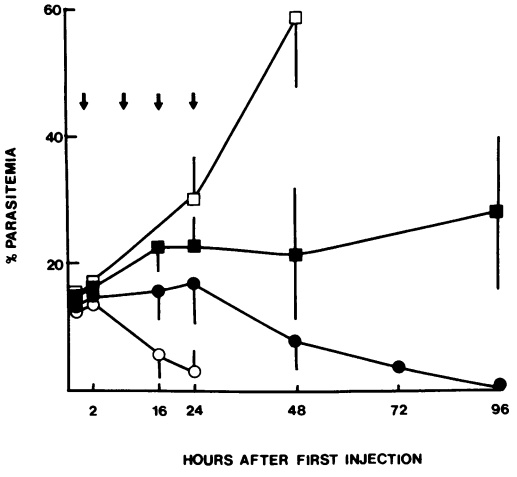

FIG. 4. Effect of repeated doses of phenylhydrazine or hydrogen peroxide on mice infected with *P. vinckei*. Symbols: (●) subcutaneous phenylhydrazine (30 mg/kg); (○) subcutaneous phenylhydrazine (60 mg/kg); (■) intravenous hydrogen peroxide (200 μl of 1% [vol/vol]); (□) controls. Standard deviations are indicated by vertical bars; n = 5 for each group. Representative outcome of three experiments is shown. Times of injection are indicated by arrows.

on these other sites since erythrocytes (28) and vascular endothelial cells (25) are particularly vulnerable to oxygen toxicity.

From other reports of the use of desferrioxamine (21), DDC (19), and propanol (23) in conjunction with alloxan, our results with these agents (Fig. 2 and 3) were consistent with the alloxan-induced hemolysis and parasite death being caused by reactive oxygen species, probably hydroxyl radicals. Murray and Cohn have argued that another protozoan parasite, *Toxoplasma gondii*, is susceptible to hydroxyl radicals, the product of superoxide and hydrogen peroxide, rather than the reactants themselves (34).

Our results with glucose pretreatment (Fig. 2) render it unlikely that alloxan kills intraerythrocytic parasites by successfully competing with glucose, for which the parasitized erythrocyte has a high requirement. This view is reinforced by our experiments with desferrioxamine, an agent we would not expect to block glucose competition, but which blocks the action of alloxan against the parasites (Fig. 2).

Interpretation of our alloxan results to mean that *P. vinckei* is susceptible to increased in vivo generation of reactive oxygen species is strengthened by the experiments in which phenylhydrazine and hydrogen peroxide caused intraerythrocytic suppression of this parasite (Fig. 4). This is consistent with an earlier report that phenylhydrazine damages intraerythrocytic *Plasmodium knowlesi* in rhesus monkeys (41). As with alloxan (Fig. 3), both of these agents produced hemoglobinuria in malarial mice, but not in controls. There is ample evidence that phenylhydrazine-induced hemolysis is mediated by free oxygen radicals (17, 24). Since in our system both this hemolysis and that induced by hydrogen peroxide were inhibited by iron chelation, the arguments used for alloxan-induced hemolysis being mediated by hydroxyl radicals may also apply to phenylhydrazine and hydrogen peroxide itself.

As well as their established role in killing bacteria ingested by phagocytes, free oxygen radicals have recently been demonstrated to kill several species of protozoa which parasitized macrophages (33, 34, 36). Since these radicals can traverse erythrocyte membranes (29) and may be released by phagocytes, as well as act within them (37), it seems reasonable to predict that intraerythrocytic protozoa, such as malaria parasites, may sustain damage as their host erythrocytes move between fixed macrophages secreting toxic oxygen species. The small blood vessels of the liver and spleen provide ample opportunity for this interaction. We suggest that macrophages in *Mycobacterium bovis* BCG- or *Propionibacterium acnes*-treated mice, which release toxic oxygen species after appropriate triggering (37), may kill *P. vinckei* in circulating erythrocytes in this way. This could contribute to the host protection and associated intraerythrocytic death of parasites that we have observed under these conditions (4, 5).

Infection with *P. vinckei* and parenteral *M. bovis* BCG or *P. acnes* produces similar functional changes in macrophages and primes them to release the same array of mediators (6). Thus, oxidizing free radicals could be released from macrophages and monocytes during the crisis of a malarial infection and harm parasites, erythrocyte membranes, and endothelial cells. This could contribute to the hemolysis, cerebral symptoms, and pulmonary and renal insufficiency which can occur in *P. falciparum* malaria in humans. Endothelial damage has been described in experimental cerebral malaria (40), and the functional changes of the pulmonary insufficiency of severe *P. falciparum* malaria (13) are consistent with this lesion. In addition, the predisposition of the erythrocytes in malarial mice to alloxan-induced lysis (Fig. 3) suggests that these cells are already under oxidative stress. This implies that oxidizing free radicals are released in undisturbed malarial infections.

The antimalarial drugs dapsone and primaquine exert oxidative pressure on normal erythrocytes (7, 18) and cause hemolysis in glucose-6-phosphate dehydrogenase (G-6-PD)-deficient individuals. This also occurs with sulfonamides of the type used in conjunction with pyrimethamine against malaria (47). Since our results indicate that *P. vinckei* is very susceptible to one of the reactive oxygen species, they raise the possibility that the oxidative capacity of dapsone and primaquine may contribute to their antimalarial activity.

Our results are consistent with the experiments of Friedman (15), who found that the sensitivity of *P. falciparum* to high oxygen tensions was increased in thalassemic and G-6-PD-deficient erythrocytes in vitro with protection by tocopherol. Thus, since *P. falciparum* also appears to be susceptible to oxidizing free radicals, any alteration in erythrocyte biochemistry which leads to erythrocytes sustaining increased oxidative damage might be expected, to the host's advantage, to inhibit the intraerythrocytic stages of this parasite. Such alterations are conferred by a range of genes common in past or present malarial areas (32); thalassemic erythrocytes are more susceptible than normal erythrocytes to both autoxidation and exposure to peroxide (29, 45, 46), and sickled erythrocytes contain less than normal glutathione peroxidase and catalase and show evidence of oxidative stress (9). These cells are also reported to contain only one-half of the normal level of the

antioxidant tocopherol (27). A further example is provided by the poor growth of *P. falciparum* in erythrocytes with the hemoglobin E mutation (35). This hemoglobulin is unstable, exposing the erythrocyte to increased oxidative stress (16). Likewise, hereditary persistence of fetal hemoglobin, a genetically determined trait, has been associated with resistance to *P. falciparum*, with retarded parasite development in erythrocytes containing fetal hemoglobin (38). This form of hemoglobin more readily transforms to methemoglobin (50), a process which generates superoxide (31), than does the form found in adults. Also, as noted by Friedman (15), the limited degradation of hydrogen peroxide in G-6-PD-deficient erythrocytes (48) would increase oxidative pressure on any intraerythrocytic parasite. Thus, it is conceivable that at least part of the apparent survival advantage of all five of these mutations in malarial areas hinges on susceptibility of *P. falciparum* to oxidative stress. Our results with alloxan-induced hemolysis (Fig. 2) imply that during a malaria infection this stress is increased on all erythrocytes. This would be particularly felt, to the detriment of the parasite, in those genetically defective erythrocytes with a limited capacity to cope with these pressures.

Finally, we note that if this work extends as predicted, it implies that desferrioxamine, a drug already in clinical use, would be useful to arrest malarial pathology arising from damage to vascular endothelium or erythrocytes. It might also arrest the hemolysis of G-6-PD-deficient individuals given primaquine or dapsone. In addition, the remarkable in vivo activity of alloxan against *P. vinckei* might provide leads in the development of new antimalarial drugs. Its rapid action through a pathway involving peroxide generation suggests that alloxan could be a model for understanding how Qinghaosu, a new drug currently under investigation (11), acts against malaria parasites.

ACKNOWLEDGMENT

This investigation received support from the malaria component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

LITERATURE CITED

1. **Carson, P. E., C. L. Flanagan, C. E. Ickes, and A. S. Alving.** 1956. Enzyme deficiency in primaquine-sensitive erythrocytes. Science **124**:484–485.
2. **Clark, I. A.** 1978. Does endotoxin cause both the disease and parasite death in acute malaria and babesiosis? Lancet **i**:75–77.
3. **Clark, I. A.** 1981. Thromboxane may be important in the organ damage and hypotension of malaria. Med. Hyp. **7**:625–631.
4. **Clark, I. A., A. C. Allison, and F. E. G. Cox.** 1976. Protection of mice against *Babesia* and *Plasmodium* with BCG. Nature (London) **259**:309–311.
5. **Clark, I. A., F. E. G. Cox, and A. C. Allison.** 1977. Protection of mice against *Babesia* spp. and *Plasmodium* spp. with killed *Corynebacterium parvum*. Parasitology **74**:9–18.
6. **Clark, I. A., J.-L. Virelizier, E. A. Carswell, and P. R. Wood.** 1981. Possible importance of macrophage-derived mediators in acute malaria. Infect. Immun. **32**:1058–1066.
7. **Cohen, G., and P. Hochstein.** 1964. Generation of hydrogen peroxide in erythrocytes by hemolytic agents. Biochemistry **3**:895–900.
8. **Cottrel, T. S., O. R. Levine, R. M. Senior, J. Wiener, D. Spiro, and A. P. Fishman.** 1967. Electron microscopic alterations at the alveolar level in pulmonary edema. Circ. Res. **21**:783–797.
9. **Das, S. K., and R. C. Nair.** 1980. Superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidation of normal and sickled erythrocytes. Br. J. Haematol. **44**:87–92.
10. **Degowin, R. L., R. G. Eppes, R. D. Powell, and P. E. Carson.** 1966. The haemolytic effects of diaphenylsulphone (DDS) in normal subjects and in those with glucose-6-phosphate-dehydrogenase deficiency. Bull. W.H.O. **35**:165–197.
11. **Editorial.** 1982. Qinghaosu: a new antimalarial. Br. Med. J. **284**:767–768.
12. **Fee, J. A., and H. D. Teitelbaum.** 1972. Evidence that superoxide dismutase plays a role in protecting red blood cells against peroxidative hemolysis. Biochem. Biophys. Res. Commun. **49**:150–158.
13. **Fein, A., E. C. Rackow, and L. Shapiro.** 1978. Acute pulmonary edema in *Plasmodium falciparum* malaria. Am. Rev. Respir. Dis. **118**:425–429.
14. **Fischer, L. L., and S. A. Hamburger.** 1980. Inhibition of alloxan action in isolated pancreatic islets by superoxide dismutase, catalase, and a metal chelator. Diabetes **29**:213–216.
15. **Friedman, M. J.** 1979. Oxidant damage mediates variant red cell resistance to malaria. Nature (London) **280**:245–247.
16. **Frischer, H., and J. Bowman.** 1975. Hemoglobin E, an oxidatively unstable mutation. J. Lab. Clin. Med. **85**:531–539.
17. **Goldberg, B., A. Stern, and J. Peisach.** 1976. The mechanism of superoxide anion generation by the interaction of phenylhydrazine with hemoglobin. J. Biol. Chem. **251**:3045–3051.
18. **Goldstein, B. D., and E. M. McDonagh.** 1976. Spectrofluorescent detection of in vivo red cell lipid peroxidation in patients treated with diaminodiphenylsulphone. J. Clin. Invest. **57**:1302–1307.
19. **Grankvist, K.** 1981. Alloxan-induced luminol luminescence as a tool for investigating mechanisms of radical-mediated diabetogenicity. Biochem. J. **200**:685–690.
20. **Grankvist, K., S. Marklund, J. Sehlin, and I.-B. Täljedal.** 1979. Superoxide dimutase, catalase and scavengers of hydroxyl radical protect against the toxic action of alloxan on pancreatic islet cells in vitro. Biochem. J. **182**:17–25.
21. **Gutteridge, J. M. C., R. Richmond, and B. Halliwell.** 1979. Inhibition of the iron-catalyzed formation of hydroxyl radicals from superoxide and of lipid peroxidation by desferrioxamine. Biochem. J. **184**:469–472.
22. **Heikkila, R. E., and F. S. Cabbat.** 1982. The prevention of alloxan-induced diabetes in mice by the iron chelator detapac. Experientia **38**:378–379.
23. **Heikkila, R. E., B. Winston, G. Cohen, and H. Barden.** 1976. Alloxan-induced diabetes—evidence for hydroxyl radical as a cytotoxic intermediate. Biochem. Pharmacol. **25**:1085–1092.
24. **Jain, S. K., and P. Hockstein.** 1979. Generation of superoxide radicals by hydrazine and its role in phenylhydrazine-induced hemolytic anemia. Biochim. Biophys. Acta **586**:128–136.
25. **Kistler, G. S., P. R. B. Caldwell, and E. R. Weibel.** 1967. Development of fine structural damage to alveolar and capillary lining cells in oxygen-poisoned rat lungs. J. Cell Biol. **32**:605–628.

26. **Kontos, H. A., E. P. Wei, W. D. Dietrich, R. M. Navari, J. T. Povilshock, N. R. Ghatak, E. F. Ellis, and J. L. Patterson.** 1981. Mechanism of cerebral arteriolar abnormalities after acute hypertension. Am. J. Physiol. **240**:511–527.
27. **Lubin, B., and D. Chiu.** 1980. Abnormal susceptibility of sickle erythrocytes to lipid peroxidation, p. 159–162. *In* S. K. Srivastava (ed.), Red blood cell and lens metabolism. Elsevier/North Holland, New York.
28. **Lynch, R. E., and I. Fridovich.** 1978. Effects of superoxide on the erythrocyte membrane. J. Biol. Chem. **253**:1838–1845.
29. **Lynch, R. E., and I. Fridovich.** 1978. Permeation of the erythrocyte stroma by superoxide radical. J. Biol. Chem. **253**:4697–4699.
30. **McDaniel, M. L., C. E. Roth, C. J. Fink, and P. E. Lacy.** 1976. Effect of anomers of D-glucose on alloxan inhibition of insulin release in isolated perfused pancreatic islets. Endocrinology **90**:535–540.
31. **Misra, H. P., and I. Fridovich.** 1972. The generation of superoxide radical during autoxidation of hemoglobin. J. Biol. Chem. **247**:6960–6962.
32. **Motulsky, A. G.** 1960. Metabolic polymorphisms and the role of infectious diseases in human evolution. Human Biol. **32**:28–62.
33. **Murray, H. W.** 1981. Susceptibility of *Leishmania* to oxygen intermediates and killing by normal macrophages. J. Exp. Med. **153**:1302–1315.
34. **Murray, H. W., and Z. A. Cohn.** 1979. Macrophage oxygen-dependent antimicrobial activity. I. Susceptibility of *Toxoplasma gondii* to oxygen intermediates. J. Exp. Med. **150**:938–949.
35. **Nagel, R. L., C. Raventos-Suarez, M. E. Fabry, H. Tanowitz, D. Sicard, and D. Labie.** 1981. Impairment of growth of *Plasmodium falciparum* in HbEE erythrocytes. J. Clin. Invest. **68**:303–305.
36. **Nathan, C., N. Nogueira, C. Juangbhanich, J. Ellis, and Z. Cohn.** 1979. Activation of macrophages in vivo and in vitro. Correlation between hydrogen peroxide release and killing of *Trypanosoma cruzi*. J. Exp. Med. **149**:1056–1068.
37. **Nathan, C. F., and R. K. Root.** 1977. Hydrogen peroxide release from mouse peritoneal macrophages. Dependence on sequential activation and triggering. J. Exp. Med. **146**:1648–1662.
38. **Pasvol, G., D. J. Weatherall, and R. J. M. Wilson.** 1977. Effects of foetal haemoglobin on susceptibility of red cells to *Plasmodium falciparum*. Nature (London) **270**:171–173.
39. **Rachmilewitz, E. A., B. H. Lubin, and S. B. Shohet.** 1976. Lipid membrane peroxidation in β-thalassemia major. Blood **47**:495–505.
40. **Rest, J. R., and D. H. Wright.** 1979. Electron microscopy of cerebral malaria in golden hamsters (*Mesocricetus auratus*) infected with *Plasmodium berghei*. J. Pathol. **127**:115–120.
41. **Rigdon, R. H., D. W. Micks, and D. Breslin.** 1950. Effect of phenylhydrazine hydrochloride on *Plasmodium knowlesi* infection in the monkey. Am. J. Hyg. **52**:308–322.
42. **Rose, C. S., and P. György.** 1950. Hemolysis with alloxan and alloxan-like compounds, and the protective action of tocopherol. Blood **5**:1062–1067.
43. **Rose, C. S., and P. György.** 1952. Specificity of hemolytic reaction in vitamin E-deficient erythrocytes. Am. J. Physiol. **168**:414–420.
44. **Smith, M. E., R. Gunther, M. Gee, J. Flynn, and R. H. Demling.** 1981. Leukocytes, platelets, and thromboxane A_2 in endotoxin-induced lung injury. Surgery **90**:102–107.
45. **Stocks, J., M. Kemp, and T. L. Dormandy.** 1971. Increased susceptibility of red blood cell lipids to autoxidation in haemolytic states. Lancet **i**:266–269.
46. **Stocks, J., E. L. Offerman, C. B. Modell, and T. L. Dormandy.** 1972. The susceptibility to autoxidation of human red cell lipids in health and disease. Br. J. Haematol. **23**:713–724.
47. **Tarlov, A. R., G. D. Brewer, P. E. Carson, and A. S. Alving.** 1962. Primaquine sensitivity. Arch. Intern Med. **109**:209–234.
48. **Tarlov, A. R., and R. W. Kellermeyer.** 1961. The hemolytic effect of primaquine. XI. Decreased catalase activity in primaquine-sensitive erythrocytes. J. Lab. Clin. Med. **58**:204–216.
49. **Wei, E. P., H. A. Kontos, W. D. Dietrich, J. T. Povlishock, and E. F. Ellis.** 1981. Inhibition by free radical scavengers and by cyclooxygenase inhibitors of pial arteriolar abnormalities from concussive brain injury in cats. Circ. Res. **48**:95–103.
50. **Wind, M., and A. Stern.** 1977. Comparison of human adult and fetal hemoglobin: aminophenol-induced methemoglobin formation. Experientia **33**:1500–1501.